

February 11, 1954 9 Feb. 15

I. Review of previous discussion

1. Began consideration of the change at a normal **C** gene to give a mutable condition -- from recessive, colorless, c, to full color, C.

2. Condition found in 1 of 3500 tested gametes from an single plant whose constitution was:

<u>C Sh wx Ds</u>	<u>Ac</u>
C Sh wx Ds	ac

3. It was shown that mutations to C occur only in presence of Ac

4. Without Ac, the colorless phenotype, recessive, completely stable:
When Ac added to nucleus by appropriate cross, mutations commence.

II. The stability of the C mutant from c-ml:

1. The mutant areas in c/c/c-ml kernels:

2. The germinal mutations: How formed:

c/c-ml Ac ac Crossed to c/c: Few fully colored kernels.

These come from mutations at c-ml before gamete formation.

3. Tests of the stability of the C mutants:

a) Test type 1: Single kernel, from above cross.

Self-pollinated for transmission of C mutant and homozygous condition.

Crossed by Ac-tester to determine presence and absence of Ac

Crossed to c / c, ds ac -- to test for C phenotype.

Crossed to c / c Ac. -- to test for reaction of C in presence of Ac, should it be absent from the plant being tested.

Results: The C action completely stable in the presence of Ac.

b). Test type 2 - progeny test from a single germinal mutation carrying kernel.

Self-pollinated ear derived from plant with mutant ^c: Kernels of all types selected; Tested as above.

Results: The same as above. Mutnat C as stable as C from which it was derived.

III. The presence of Ds in the c-ml carrying chromosome.

1. Original cross: ygc sh wx ds ac female ~~xx~~ YgC Sh wx Ds Ac male
YgC Sh wx Ds ac

2. The appearance of plant from this kernel: Streaks of yg -- Ds type pattern.

3. Constitution of plant was:

$\frac{Yg \ c \ Sh \ wx}{yg \ c \ sh \ wx \ ds}$	$\frac{Ac}{ac}$
--	-----------------

4. This plant crossed to $yg \ c \ sh \ \overset{Wx}{/} \ yg \ c \ sh \ no \ Ds$, no Ac

5. The c to C var. kernels in Sh Wx class removed. Plants grown from them.

The c Sh Wx non-var. kernels removed.

The c sh Wx " " " "

6. The green plants (Yg/yg plants) in each of these sub-cultures compared.

a). All plants coming from the c to C kernels showed Yg to yg var.

b). None of the Yg/yg plants coming from the colorless Sh Wx kernels showed var. for yg.

c). This would suggest that no Ds was at standard location in c-ml parent. (Crossovers should be present in the c Sh Wx and c sh Wx classes introducing Ds. These not found.)

7. The plants derived from the c to C kernels crossed to c sh wx ds ac female

a). c sh wx ds ac female	x	$\frac{c-ml \ Sh}{c \ sh}$	$\frac{wx}{Wx}$	$\frac{Ac}{ac}$
--------------------------	---	----------------------------	-----------------	-----------------

b). The types of kernels on the ear: Expected appearance

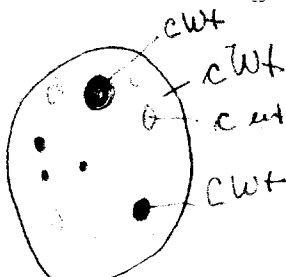
non-crossovers	c Sh wx Ac	c to C var.
	c Sh wx no Ac	colorless Sh wx

Crossovers, region 1	c-ml sh Wx Ac	c to C sh Wx
	c-ml sh Wx ac	colorless sh Wx

Crossovers, region 2	c-ml Sh Wx Ac	c to C Sh Wx
	c-ml Sh Wx ac	c Sh Wx

the c sh Wx chromosome -- non crossovers	c sh Wx
c Sh wx crossover reg. 1	c Sh wx
c sh wx " " 2	c sh wx

c). Appearance of kernels having c-ml and Wx: (Crossovers)



- d). No wx areas found in any of the colorless Sh or sh Wx kernels.
 - e). In the variegated kernels, the C areas are all Wx (exceptions to be taken up later). Ds activity no longer evident when mutation to C occurs.
 - f). This is like the tests of germinal mutations to C -- no Ds activity found to occur in chromosomes 9 with these mutants.
8. Plants that ~~were~~ c-ml Sh wx / c sh wx Ac/ ac (original plant) also crossed to C sh bz ds ac females:

Kernels types:

9. Conclusions:

- a). Ds close to or at the locus of C -- that is, c-ml
- b). No Ds at the standard location in original plant.
- c). Mutations to C associated with loss of Ds activity at the C locus.
- d). No crossovers between c-ml and Ds observed. No kernels found with Ds activity without c to C mutations.
- e). Mutations to C occur only when Ac present.
- f). Can begin to consider the possibility that c-ml arose through transposition of Ds to the C locus.

CDs -- colorless; action of C inhibited.

Loss of Ds from this locus -- return of C action.

IV. Attempts to remove Ds by crossing over -- to get a "mutation to C"

1. The test: yg c sh wx ds ac female x $\frac{Yg\ c-ml\ Sh\ Wx}{yg\ c\ sh\ wx}\ \frac{ac}{ac}$ male

~~Plants~~ crossed to Ac tester
crossed to c Ac to test for c-ml

20,000 kernels from the test cross -- none showed any C color,

V. Attempts to remove Ds from C by breakage-fusion-bridge cycle. This a small test. Not many kernels analysed.

1. The cross: $\text{Re } c \text{ sh } wx \text{ ds } \underline{\text{ac}} \text{ female } \times \text{ c-m1 Sh Wx } \underline{\text{ac}} \text{ male.}$

2. Meiotic prophase pairing:



3. The crossovers -- the anaphase bridges:



4. The transmissible gametes: $\times \text{ c-m1 sh W+}$

5. The results: No cases found where color appeared. Small test. Larger test probably would have shown this -- formation of a new Ac factor.

sh W+ kernels showing W+ = w+ cor.

6. Conclusions: Ds must be very close to the C gene possibly a component of it,

or

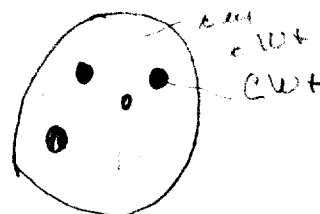
Presence of Ds alters crossing-over: Reduced here.

Whatever the explanation, the DsC situation acts as a unit.

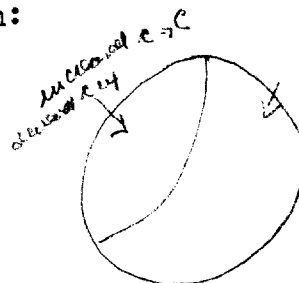
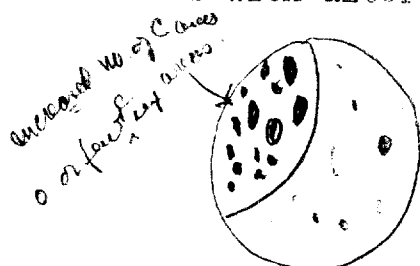
Changes in state of c-m1

1. The original state of c-m1. Appearance of kernels with:

$c \text{ wx}/c \text{ wx}/ \text{ c-m1 Wx, Ac ac ac}$



The sectors with altered action:



Found few kernels with whole area like one of the described sectors.

2. Plants grown from the selected kernels with altered relationships between number of mutations to C and number of spots of wx. Each kernel carefully examined and pattern analysed.

a). Range of selections:

From very much increased frequency of c to C mutations and no wx areas to those in which there were only a few C specks and many wx areas.

3. Each plant derived from these kernels crossed to $\frac{yg}{c}/\frac{yg}{ac}$ female plants.

Variegated kernels on resulting ear compared with each other and with pattern appearing on the kernel from which plant arose. in kernel

The result: the pattern of variegation resembled that/ from which the plant arose. This very constant for those cases where the rate of c to C mutations were high and the rate of appearance of wx areas were less.

Plants grown from the variegated kernels in each of the test types:

Constitutions: $\frac{Yg}{yg} \frac{c-ml}{c} \frac{\text{centromere}}{ds \text{ centromere}} Ac \text{ ac}$

4. The appearance of the plants with regard to Yg to yg variegation: This a direct test for frequency of breaks at c-ml. Do not have to rely on b. f. b. cycles as with the kernels. Each breaks eliminates Yg

Plant variegation

Kernel type

Green; seldom if any yg streaks

Many c to C mutations; no wx areas

Green; few yg streaks

High frequency of c to C mutations; few wx areas

Green; many yg streaks

Lower frequency of c to C mutations; higher frequency of wx spots

very Green; many yg streaks. heavily streaked.

Low frequency of c to C mutations; high frequency of wx spots

5. Conclusions:

(1) Some change has occurred to the that give c to C mutations without chrdicentric formations.

(2). The total number of events at th changed. It is the consequence of the frequency.

(3). The alterations of DsC that giv suddenly -- and only when Ac is presen

(4). These changes at the locus are

DsC unit that alters the ratio of events omosome breaks to those that give

is DsC locus does not appear to have event that is altered in ratio of

e rise to these changed conditions arose t. called -- changes in state of the locus,

II. The stability of the different altered states of c-ml.

1. Changes in state occur relatively frequently if the state under consideration gives many dicentric chromatids as the consequence of an event at the DSC locus.

2. They are rare if the state under consideration gives few dicentrics

3. If the state gives no dicentrics, or exceedingly few of them, the state is very stable. It does not change with regard to occurrence of breaks -- that is, dicentric chromatids. (Emphasize the dicentric aspect)

a). This is important. It results in a "pure" mutable gene. This quite comparable to other known mutable genes where breaks apparently do not occur or are rare.

b). This state of c-ml is comparable in its behavior in all major respects to the a_1 - Dt condition, long known in maize.

III. The state of Ds at its standard location illuminated by the study of changes in state of c-ml.

1. The sudden change in action of Ds at its standard location -- Ds^{fl} described earlier:

2. Tests made of its stability: Quite stable. Had to make very rigid selections among many kernels to obtain a state with increased frequency of dicentric formations.

3. The increments in increase -- occurred stepwise.

4. The plants -- comparisons of variegation patterns when constitutions:

$\frac{Yg \ I \ D_s}{Yg \ c \ ds}$ Ds-standard location	$\frac{Ac}{ac}$	$\frac{Yg \ c-ml \ Wt}{Yg \ c \ Wt}$	$\frac{Ac}{ac}$
Appearance of kernel	Appearance of plant from kernel	Appearance of kernel	
many areas of $c \ ds$ →	very many yg streaks ←	few $c \rightarrow C$ mutations; many ac areas	
fewer " " " →	fewer yg " ←	increased no $c \rightarrow C$ mutations; fewer ac areas	
few " " Ds ^{fl} →	very few yg " ←	many $c \rightarrow C$ mutations; very few ac areas	
	no yg streaks; only an occasional	very many $c \rightarrow C$ mutations; no ac areas	

5. Conclusions:

a). The Ds^{fl} state reflects same condition as at c-ml with state having few dicentric forming events in comparison with mutations to C.

b). The Ds^{fl} pattern -- reflects only those events at Ds that give rise to dicentrics. Probably as many other changes occur as with c-ml but the result can not be identified by a phenotypic alteration, as with c-ml. Possibly some way could be found to detect these phenotypic changes, but not an easy one.

→ *Begin Feb. 18*

IV. The origin of stable recessives, c, from c-ml

1. The test: c-ml/c-ml, Ac/Ac (allelic) female x c/c/ ad/ac male

2. The ear: Few colorless, non variegated kernels appeared on some ears.

a). These could arise from changes of Ac and losses of

b). From changes of c-ml to give a stable c.

3. Some of the colorless kernels selected: Most resulted from changes of Ac. A few from changes of c-ml to give stable c, or no c to C mutations in the tests made.

The tests: Tests for presence of Ac -- Ac tester stock

Crossed to c/c/ with Ac to test action of c-ml

Crossed to c/ c no Ac.

4. The stable c from c-ml:

a). No c to C mutations (within tests made) when Ac present.

b). No dicentric formations. -- no breaks at c

c). A new stable, recessive c mutant. Much like those stable a_1 mutants obtained from a_1 -Dt tests.

The action of Ac on c-ml and Ds-standard location when both in same kernel.

1. The cross: c-ml wx ds; Ac female x c Wx Ds ac male.

State of c-ml
High frequency of c to C mutations
No dicentrics or rare.

State of Ds: High frequency
of dicentric formations

2. The constitution of the kernels:

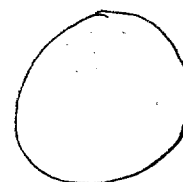
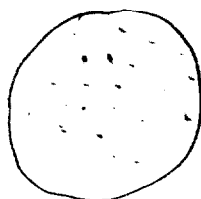
c-ml wx / c-ml wx / c Wx Ds; Ac Ac ac

Background of kernel: colorless aleurone; Wx in starch of underlying cells.

- a). Consequence of a break at Ds -- a wx area
 - b). Consequence of a mutation at c-ml -- a C area in the aleurone layer.
3. The general appearance of the kernels:

The color in the aleurone layer

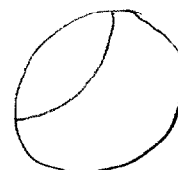
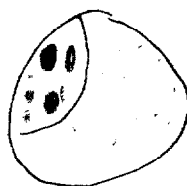
The wx spots in the underlying cells



4. The changes occurring somatically to Ac to produce sectors with altered action -- lower dose type or increased dosage type:

Lower dose sector

No Ac action sector



5. The coincident events at c-ml and at Ds-standard: What to expect:



What observed: Many coincidences observed. Some without coincidence. Difficulties in making exact interpretation:

- a). Changes in state of Ds as consequence of this event.
- b). Translocations, etc, instead of a dicentric formation -- occur. Then, no wx spot need be formed.

The control of Ac on the time and the cells in which events at both c-ml and Ds at standard location will occur:

1. The doses of Ac -- and easily seen relationship, as shown above.
2. The responses of both to the stabilized Ac.

C sh bz wx ds ac x I Sh Bz Wx Ds
Stabilized Ac

c ds ac x c-ml
stabilized Ac

Photo (1), (2)



The Transposition of Ds from the locus of c-ml

1. How recognized: In sectors that are C; in the kernels c/c/c-ml, Ac, ac ac

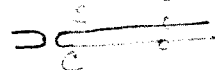
Ds to right of C



Ds to left of C
Twin-spots



B.F. f cycles:



2. The occasional appearance of germinal mutation with Ds in new position: in crosses of c female by c-ml Ac male.

To right of C

To left of C

Twin-spots

white
brown like sector

white brown
brown sector

3. The tests of the plants derived from these kernels:

a). Crossed to c/c ac females

b). Kernels on ear: ~~xxxxxxxx~~ Show that Ds is present in C carrying chromosome: Only about 5 or 6 cases examined. Can remember the positions of only three of them:

1) C Ds Sh for one

2) Ds C Sh for another

3) Ds very close to C for the fourth - no twin spots.

gave some new mutables. seemed unstable. C - a +
Reason for this became quite apparent in c → C kernel.
another study - The spread of mutations change along the chromosome.